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Activity of entomopathogenic nematodes (Rhabditida) against cereal leaf beetle (*Oulema melanopus* [L.], Coleoptera, Chrysomelidae) adults under laboratory conditions

Žiga LAZNIK[1], Melita ŠTRUKELJ[2], Stanislav TRDAN[3]

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ABSTRACT

In 2009, three Slovenian strains of entomopathogenic nematodes) and commercial product Entonem (active ingredient *S. feltiae*), were tested under laboratory conditions for their activity against adult cereal leaf beetles (*Oulema melanopus*). The nematode strains were tested at four different doses (250, 500, 1000, and 2000 infective juveniles/adult) and at three temperatures (15, 20, and 25 ºC). *Steinernema carpocapsae* strain C101 was the most effective and showed itself to be a good alternative to chemical insecticides, and appears to have the highest potential for controlling overwintered cereal leaf beetles under field conditions. In our bioassay the temperature had the greatest influence on the efficacy of the entomopathogenic nematode strains; both *S. feltiae* treatments (strain B30 and Entonem) proved to work better at the lowest temperature, however the strain *H. bacteriophora* D54 had its best efficacy at the highest temperature in the experiment. Several species *(S. feltiae* and *S. carpocapsae*) have been efficient at lower suspension concentrations, which enables their economical usage against the cereal leaf beetle in integrated cereal production in the future.

Key words: entomopathogenic nematodes, *Oulema melanopus*, biological control, laboratory experiment

IZVLEČEK

DELOVANJE ENTOMOPATOGENIH OGORČIC (Rhabditida) NA ODRASLE OSEBKE RDEČEGA ŽITNEGA STRGAČA (*Oulema melanopus* [L.], Coleoptera, Chrysomelidae) V LABORATORIJSKIH RAZMERAH

V letu 2009 smo v laboratorijskem poskusu preizkušali učinkovitost treh domačih ras entomopatogenih ogorčic in komercialnega pripravka Entonem (aktivna snov *S. feltiae*) zoper odrasle osebke rdečega žitnega strgača (*Oulema melanopus*). Delovanje entomopatogenih ogorčic smo preizkušali pri štirih različnih koncentracijah (250, 500, 1000 in 2000 infektivnih ličink/osebek) in treh različnih temperaturah (15, 20 in 25 ºC). Rasa C101 vrste *Steinernema carpocapsae* je bila najbolj učinkovita in bi lahko predstavljala dobro alternativo kemičnim insekticidom pri zatiranju prezimljenih odraslih osebkov rdečega žitnega strgača na prostem. V našem poskusu je imela največji vpliv na delovanje entomopatogenih ogorčic temperatura; obe obravnavanji z vrsto *S. feltiae* (rasa B30 in Entonem) sta bili učinkoviti tudi pri nižjih temperaturah, rasa D54 vrste *H. bacteriophora* pa je najbolje delovala pri najvišji temperaturi v poskusu. Ogorčici *S. feltiae* in *S. carpocapsae* sta zadovoljivo učinkovali tudi pri nižji koncentraciji suspenzije ogorčic, kar omogoča večjo gospodarnost rabo njihove uporabe pri zatiranju odraslih osebkov rdečega žitnega strgača v integrirani pridelavi žit v prihodnje.

Key words: entomopatogene ogorčice, *Oulema melanopus*, biotično varstvo, laboratorijski poskus

[1] Young researcher, B. Sc., University of Ljubljana, Biotechnical Faculty, Dept. of Agronomy, Chair of Phytomedicine, Agricultural Engineering, Crop Production, Grassland and Pasture Management, Jamnikarjeva 101, SI-1111 Ljubljana, e-mail: ziga.laznik@bf.uni-lj.si

[2] B. Sc., ibid.

[3] Assoc. Prof., Ph. D., ibid.

1 INTRODUCTION

In Central Europe seven chrysomelid beetles belong to the genus *Oulema* (Chrysomelidae family). Two of them, the cereal leaf beetle (CLB), *Oulema melanopus* (L.) and *O. gallaeciana* (Heyden), are pests of various cereals (Ulrich *et al*. 2004). The CLB is spread across Europe, the Middle East and Asia and in North America (Haynes and Gage, 1981; Olfert *et al*. 2004). The life history and biology of CLB is well known (Casagrande *et al*., 1977). The adults hibernate gregariously in the soil, such as in field debris, in the crevices of tree bark, or inside rolled leaves (Casagrande *et al*., 1977). The adults become active in the spring, when temperature reaches 10 ºC, and feed initially on wild grasses. Oviposition begins about 14 days after adults resume activity in the spring. During the following two months each female lays several hundred eggs. The larvae pass through four instars, each lasting two to three days. Pupation occurs in the soil up to five cm beneath the surface. The species are univoltine. Adults feed but become less and less active during the summer and early autumn. The larvae and adults feed on the upper layer of green mesophyll cells, down to the cuticle, staying between the leaf veins (Ulrich *et al*., 2004). This feeding pattern is characteristic of the CLB and is one way of detecting its presence (Campbell *et al*., 1989).

The economic impact of the CLB can be significant. Heyer (1977) estimated that a single larva reduces assimilation by about 10 %. A massive attack of larvae reduces total assimilation by up to 80 % (Grala *et al*., 1991) causing losses of about one tonne of grain per ha. Previous studies recommended an economic threshold of one larva per stem (Haynes and Gage, 1981), but this infestation level often results in unacceptably high levels of defoliation (Buntin *et al*., 2004). More recent studies have suggested a much lower economic threshold (Herbert and van Duyn, 1999). Pre-harvest efforts to control CLB are primarily based on the release of natural enemies; the most successful have been the egg parasite *Anaphes flavipes* (Foerster) (Maltby *et al*., 1971) and the larval parasitoid *Tetrastichus julis* (Walker) (Haeselbarth, 1989).

The application of entomopathogenic nematodes (EPNs) as biological control agents in protected environments is well documented (Kaya and Gaugler, 1993). EPNs carry species specific symbiotic bacteria which, after nematodes infect insect hosts, are released into the hemolymph of the host (Gaugler, 2002). Only infective juveniles (IJs) are able to infect the insect host (Kaya, 2000). Research has demonstrated that EPNs at high concentrations, together with favourable abiotic factors (high humidity, optimal temperature) can be effective biological control agents of adult chrysomelids (Journey and Ostlie, 2000; Trdan *et al*., 2008). Recent research has confirmed their efficacy in controlling adult western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (van der Burgt *et al*., 1998; Toepfer *et al.,* 2005), flea beetles (*Phyllotreta* spp.) (Trdan *et al*., 2008), and the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Campos-Herrera and Gutiérrez 2009; Trdan *et al*., 2009). Since adult CLB are found in protected areas we hypothesize that they should be particularly susceptible to EPN infections.

The aim of our research was to study the activity of entomopathogenic nematodes against the CLB adults, to determine which species of EPN (*S. feltiae*, *S. carpocapsae*, *Heterorhabditis bacteriophora*) is the most effective, and to investigate how the effectiveness of EPNs is related to temperature and the nematode concentration. In practice, farmers usually do not control the adult stages of CLB, although it was established in one research study that the most effective treatments were low rates of lambda cyhalothrin when applied early while adults were still laying eggs and before or near 50% egg hatch (Buntin *et al*., 2004). With the potential efficacy of entomopathogenic nematodes with regard to CLB adults, we would acquire the results necessary for replacing insecticides with the biological control agents mentioned. The most efficient strain shown by our research would then be suggested for incorporation in a sustainable strategy of cereals production. In this way we would contribute to more environmentally friendly production of cereals.

2 MATERIALS AND METHODS

2.1 Entomopathogenic nematodes and the cereal leaf beetle

The investigation was carried out during 2009 in Ljubljana (Biotechnical Faculty, Dept. of Agronomy), Slovenia. The commercial preparation (Entonem) was obtained from Koppert B.V., the Netherlands. The EPNs in this preparation is *Steinernema feltiae* (Filipjev). Once received, the nematode preparation was stored in the dark in a refrigerator (2-4 ºC). Three Slovenian isolates of EPNs were also included in the experiment. All three strains were isolated from the soil (Laznik *et al*., 2008; Laznik *et al*., 2009abc). Two Slovenian species (*S. carpocapsae* C101 and *H. bacteriophora* D54) were tested for the first time in this experiment, while *Steinernema feltiae* strain B30 has been proven to very effective in a field experiment against the Colorado potato beetle (Laznik *et al*., 2009d) and in a laboratory assay against rice weevil (Laznik *et al*., 2010). All EPN strains were reared

using late instar larvae of *Galleria mellonella* (L.) (Bedding and Akhurst, 1975). We used only infective juveniles which were less than 2 weeks old. During the experiment we stored the infective juveniles in a water suspension at 4 ºC in the refrigerator.

CLB adults were collected from a test plot of winter wheat being grown by members of the Biotechnical Faculty in Ljubljana. The CLB were caught in sweep nets in late morning, after the dew had dried. We stored the beetles after catching them in ventilated plastic containers (Trdan *et al*., 2008) and transported to the laboratory, where they were used for experimental purposes no later than 5 hours later. The adults were of different ages, replicating conditions in practice.

2.2 Laboratory bioassay

We tested the efficacy of the EPNs in controlling adults of the CLB by exposing individuals to either 0, 250, 500, 1000, or 2000 IJ/adult. We determined the number of infective juveniles in a previously prepared unknown concentration of nematode suspension by counting the number of such in droplets (5 µl x 5) and by diluting (adding tap water solution) or by concentrating (reduction to an adequate volume with the assistance of centrifugation). In this manner we obtained the selected concentrations of nematode suspensions (0, 2500, 5000, 10000, and 20000 IJ/ml).

We used the procedure described of Trdan *et al.* (2008). We placed 10 adult CLBs on a filter paper in a glass Petri dish (diameter = 9 cm) with a fresh leaf of wheat. Each treatment was repeated 10 times for a total of 100 CLB/nematode concentration. The following procedure was performed with a time interval repeated three times. One ml of each nematode concentration was added to the Petri dish which was then sealed with parafilm to prevent the beetles from escaping. The Petri dishes were put in a rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia) with out light at temperatures of 15, 20, and 25 ºC at a relative humidity of 70 %.

The number of dead adult *O. melanopus* was determined 2, 4, and 6 days after treatment (DAT). The dead individuals were dissected to determine if the nematodes were present. In such a manner we wanted to prove that the insects died due to EPN activity.

2.3 Statistical analysis

A multifactor analysis of variance (ANOVA) was conducted to determine the differences in mortality rates (%) between the adults of *O. melanopus* reared in 48 different treatments (four strains of EPNs – each with four different concentrations at three different temperatures). Before the analysis, the mean mortality was tested for the homogeneity of treatment variances. Mortality rate data were corrected for control mortality, using Abbott's formula (Abbott, 1925). The arcsine square-root was transformed before this analysis. A Student-Newman-Keuls multiple range test ($P \leq 0.05$) was used to separate mean differences among the parameters in all the treatments. For the 6 days after treatment (DAT) the values of LC_{50} and LC_{90} (the numbers of IJs/adult causing 50% and 90% mortality) were estimated, and the overall efficacy of the tested nematodes was determined from this estimates (Trdan *et al*., 2008) All statistical analyses were performed using Statgraphics Plus for Windows 4.0 (Manugistics, Rockville, MD, USA) and the figures were created with MS Office Excel 2003. The data are presented as untransformed means ± SE.

3 RESULTS

Data on analysis of the pooled results are presented in table 1.

At 15 ºC, the natural (control) mortality of the CLB adults was 0.0±0.0 % (2 DAT), 0.64±0.35 % (4 DAT) and 9.50±2.92 % (6 DAT). At 20 ºC, the comparable values were 4.82±2.16 % (2 DAT), 5.66±2.22 % (4 DAT), and 9.7±3.97 % (6 DAT). At 25 ºC, the natural mortality of the CLB adults was 2.42±1.51 % (2 DAT), 6.06±2.55 % (4 DAT) and 35.59±10.86 % (6 DAT). In all nematode treatments, mortality was greater than in the control treatments and so all of the treatments values could be corrected for the natural mortality.

Two days after treatment, the highest mortality (100 %) was recorded with the *S. carpocapsae* strain C101 kept at 20 and 25 ºC (Table 2), and the lowest with the *H. bacteriophora* strain D54 kept at 15 and 20 ºC, where the mortality at all concentrations of the nematode suspension was less than 4% (Table 2). When only *S. feltiae* strains are taken into consideration, at all three temperatures and concentrations of the nematode suspension Entonem (51 %) was more efficient than the Slovenian native strain B30 (34 %). When all strains are considered, the *S. carpocapsae* strain C101 has the highest efficacy (68 %) at the lowest temperature and at all concentrations of the nematode suspension, while only Entonem, at a concentration of 500 IJs/adult, performed equally well (62 %) (Table 2).

Table 1: ANOVA results for corrected mortality of adults of the cereal leaf beetle

Source	Adults F	df	P
DAT	1204.29	2	<0.0001*
Nematode concentration	346.56	3	<0.0001*
EPN strain	1398.56	3	<0.0001*
Temperature	233.60	2	<0.0001*
Replication in time	2.17	9	0.0703
Replication in space	0.28	2	0.7590
DAT × nematode concentration	5.96	6	0.0051
DAT × EPN strain	86.05	6	<0.0001*
DAT × temperature	72.62	4	<0.0001*
Nematode concentration × EPN strain	50.56	9	<0.0001*
Nematode concentration × temperature	58.57	6	<0.0001*
EPN strain × temperature	352.40	6	<0.0001*
DAT × nematode concentration × EPN strain	8.22	18	<0.0001*
DAT × nematode concentration × temperature	8.53	12	<0.0001*
DAT × EPN strain × temperature	25.94	12	<0.0001*
Nematode concentration × EPN strain × temperature	54.37	18	<0.0001*
DAT × nematode concentration × EPN strain × temperature	5.0	36	<0.0001*

* Source of variation significant at α=0.05

Four days after treatment, the highest mortality was recorded with Entonem (25 ºC; 2000 IJs/adult) and the *S. carpocapsae* strain C101; the latter was the most efficient at all temperatures and concentrations of the nematode suspension (Table 1). The lowest efficacy was observed with the *H. bacteriophora* strain D54 at 15 ºC, with less than 20 % efficacy on average (Table 2). Among the *S. feltiae* strains, at all three temperatures and concentrations of the nematode suspension, the efficacy of Entonem (71 %) was higher than that of the Slovenian native strain B30 (61 %). At the lowest temperature the *S. carpocapsae* strain C101 had the highest efficacy (99 %) of all the observed strains at all concentrations of the nematode suspension. At concentrations of 500 and 2000 IJs/adult at the lowest temperature, the Entonem and the Slovenian strain B30 achieved more than 80 % efficacy (Table 2).

Six days after treatment, the highest mortality was caused with the use of Entonem (25 ºC, 2000 IJs/adult), the *H. bacteriophora* strain D54 (25 ºC, all concentrations of the nematode suspension) and the *S. carpocapsae* strain C101, which caused the death of 100% of the adults of the CLB at all temperatures and concentrations of the nematode suspension (Table 2). The lowest mortality was observed with the B30 strain, which on average caused the death of 69 % of the beetles. At 15 ºC, a mortality rate of more than 90 % was achieved with the *S. carpocapsae* strain C101 and Entonem, at all suspension concentrations, and with the *S. feltiae* strain B30 at 250, 500, and 2000 IJs/adult, respectively. At the lowest temperature, the commercial and native *S. feltiae* strains performed better than at the higher two temperatures in our laboratory assay (Table 2), on the other hand, the *H. bacteriophora* strain D54 performed its best at the highest temperature (100 %).

Overall, the nematode treatments were generally more effective at 25 ºC (74 %) than at 15 ºC (64 %), and 20 ºC (60 %). Among the observed strains, C101 performed better (95 %) than the other strains included in the laboratory assay (Entonem 67 %, B30 54 %, D54 49 %). Both *S. feltiae* strains performed better at the lowest temperature (over 70 %) than at the higher temperatures, where their efficacy was only 54 %. At the highest temperature, the strain *H. bacteriophora* D54 performed better (83 %) than at the lower two temperatures (39 % and 24 %, respectively). At 2000 IJs/adult, all four nematode strains killed over 75 % of the CLB adults. Lower doses caused only from 53 % to 65 % mortality. All concentrations of the nematode suspension performed better at 25 ºC (74 %) than at 15 ºC (64 %) and 20 ºC (60 %). At 6 DAT the mortality of the CLB adults was higher (81 %) than for the other two observed days (4 and 2 DAT), where mortality was only 71 % and 48 %, respectively.

In our research we also determined LC_{50} and LC_{90} values for all four studied strains and at all three temperatures for 6 DAT, all of which are summarized in Table 3. The results showed that strain C101 had the lowest LC_{50} (2 DAT at 15 ºC: 561 IJs/adult) and LC_{90} (2 DAT at 15 ºC: 1398 IJs/adult) values at all three temperatures. The commercial product Entonem had the lowest LC_{50} and LC_{90} values at 15 ºC (422 IJs/adult and 884 IJs/adult), while strain D54 reached the lowest values of LC_{50} (4 DAT: 375 IJs/adult) and LC_{90} (4 DAT: 875 IJs/adult) at the higher temperatures (Table 3).

Table 2: Mean adult mortality (± SE) of *Oulema melanopus* adults after being treated with four different doses of four strains of EPNs and kept at 15, 20, and 25 °C. The mortality data, corrected for the control mortality, are shown for two, four, and six days after treatment.

		15 °C				20 °C				25 °C			
		Nematode concentration (IJs/adult)											
EPN strain	DAT	250	500	1000	2000	250	500	1000	2000	250	500	1000	2000
Entonem	2	46.0 ±8.7b	62.0 ±4.9c	42.0 ±3.7b	50.0 ±6.3b	29.0 ±3.2c	17.9 ±14.0b	60.5 ±0.0c	63.2 ±4.9b	3.3 ±3.3a	66.7 ±14.3b	80.6 ±7.1b	88.9 ±5.2b
	4	86.0 ±5.1b	82.0 ±7.4b	88.0 ±3.7c	94.0 ±2.5b	40.5 ±5.4b	29.7 ±11.6a	81.0 ±3.3b	78.4 ±9.2a	2.4 ±2.4a	76.5 ±10.0b	91.2 ±5.9b	100.0 ±0.0b
	6	97.3 ± 2.7bc	91.9 ±3.3b	91.9 ±3.3b	97.3 ±2.7bc	52.8 ±5.6b	52.8 ±11.3a	91.7 ±3.4b	88.9 ±6.8a	12.4 ±4.2a	85.3 ±9.3b	94.1 ±5.9b	100.0 ±0.0b
S. feltiae B30	2	6.0 ±4.0a	38.0 ±8.6b	40.0 ±4.5b	46.0 ±8.1b	4.4 ±2.6b	24.4 ±7.1b	41.5 ±4.6b	68.3 ±10.6b	35.9 ±15.5b	32.4 ±5.9a	35.3 ±5.9a	38.8 ±14.6a
	4	78.7 ±6.7b	85.1 ±2.6b	70.2 ±8.5b	87.2 ±6.2b	17.5 ±5.0a	35.0 ±4.7a	47.5 ±8.3a	85.0 ±10.0a	54.5 ±13.6b	48.5 ±3.7a	57.6 ±8.8a	63.6 ±13.2a
	6	90.5 ±6.9b	92.9 ±2.9b	83.8 ±7.1b	92.8 ±4.8b	30.6 ±6.2a	58.3 ±6.2a	61.1 ±12.0a	97.2 ±2.8ab	37.1 ±18.3b	47.6 ±4.8a	71.4 ±13.9a	71.4 ±13.8a
S. carpocapsae C101	2	34.0 ±4.0b	72.0 ±5.8d	80.0 ±4.5c	86.0 ±4.0c	87.5 ±2.1d	100.0 ±0.0c	100.0 ±0.0d	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c
	4	98.0 ±2.0c	100.0 ±0.0c	98.0 ±2.0d	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0b	100.0 ±0.0c	100.0 ±0.0b	100.0 ±0.0c	100.0 ±0.0d	100.0 ±0.0c	100.0 ±0.0b
	6	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0b	100.0 ±0.0c	100.0 ±0.0b	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0b	100.0 ±0.0b
H. bacteriophora D54	2	2.0 ±2.0a	0.0 ±0.0a	2.0 ±2.0a	0.0 ±0.0a	0.0 ±0.0a	1.6 ±1.6a	1.6 ±1.6a	3.3 ±2.0a	51.0 ±7.5b	63.3 ±8.9b	69.4 ±10.7b	40.8 ±3.8a
	4	14.0 ±5.1a	26.0 ±7.5a	12.0 ±5.8a	18.0 ±5.8a	34.1 ±6.2b	26.8 ±6.7a	51.2 ±6.7a	78.0 ±8.1a	97.1 ±2.9c	91.4 ±3.5c	94.3 ±3.5b	97.1 ±2.9b
	6	48.8 ±7.0a	60.4 ±2.9a	41.9 ±8.2a	69.8 ±10.8a	27.3 ±11.1a	72.7 ±11.1a	77.3 ±7.2a	95.4 ±4.6ab	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0b	100.0 ±0.0a

Table 3: The calculated numbers of the nematodes needed to kill 50 % (LC_{50}) and 90 % (LC_{90}) of *Oulema melanopus* adults, six days after treatment, at three different temperatures.

	Temp. (ºC)	Strain of entomopathogenic nematodes			
		Entonem	B30	C101	D54
LC_{50}[z] (95 % CL[y])	15	422 (0-2668)	866 (0-2011)	561 (254-868)[(2)]	876 (551-1201)
	20	551 (207-896)	711 (497-924)	-[(2)]	671 (386-956)
	25	664 (372-955)	877 (566-1187)	-[(2)]	375 (0-2573)[(4)]
LC_{90}[z] (95 % CL[y])	15	884 (438-1286)	938 (606-1269)	1398 (1062-1733)[(2)]	1347 (689-2004)
	20	1269 (946-1592)	1479 (1200-1758)	499 (70-927)[(2)]	1257 (957-1558)
	25	1141 (868-1414)	1228 (786-1671)	-[(2)]	875 (467-1283)[(4)]

[z] LC_{50} and LC_{90} expressed as the number of IJs per adult.
[y] Confidence limits, CL, are given in parentheses
[(2)] 100% mortality at 2 DAT
[(4)] 100% mortality at 4 DAT

4 DISCUSSION

The results of the present research have demonstrated that the mortality of CLB adults is mostly affected by temperature in connection with the concentration of the nematode suspension, different strains, and DAT. All four studied strains (B30, C101, D54, and Entonem) caused the highest mortality of CLB adults 6 days after treatment (81 %) and the highest concentration of the nematode suspension (78 %). Among the studied strains, *S. carpocapsae* C101 showed the best performance, causing a mortality rate of almost 96 % of CLB adults. On the other hand, the only *Heterorhabditis* nematode in our laboratory bioassay caused an insect mortality of only 49 %. In a comparison between *S. feltiae* nematodes, the commercial product Entonem performed better than the native strain B30 (67 % and 54 %, respectively).

At 15 and 20 ºC, lower mortality was recorded than at 25 ºC, thus supporting the results of our previous research studies (Trdan *et al*., 2006; Trdan *et al*., 2009) and the research of other groups (Belair *et al*., 2003; Yang *et al*., 2003). However both *S. feltiae* strains performed their best at the lowest temperature, which corresponds to some previous research (Williams and MacDonald, 1995; Trdan *et al*., 2009), while, on the other hand, the *H. bacteriophora* strain D54 caused the highest mortality at the highest temperature, which was also found in the results of the research of Trdan *et al.* (2008).

S. carpocapsae C101 performed the best at all three temperatures. Controlling insect pests with foliar application is becoming a more widely-used practice (Broadbent and Olthof, 1995). If this method is required for the control of the first (overwintered) adults of the CLB, the application of *S. feltiae* or *S. carpocapsae* suspensions is recommended, as our research demonstrated that this species showed the highest efficacy in controlling adults at 15 ºC. The first adults in the central and south parts of Europe usually appear in the first half of April, when the nights are still relatively fresh in the area in which our research was carried out (Stamenković, 2004).

The higher concentrations proved to be more efficient in our experiment, however all steinernematid species in the present research demonstrated sufficient efficacy also at lower concentration doses. Based on our current findings, we conclude that the activity of EPNs is influenced more by temperature than by the numbers of nematodes applied, but this tends to be species-specific (Arthurs *et al*., 2004). The minor role of the nematode concentration can be explained by the fact that only a few invasive nematodes need to penetrate an insect host in order to kill it (Bednarek and Nowicki, 1986). Our finding that several species of EPNs demonstrated the same results at lower concentrations as with higher concentrations, gives these biocontrol agents in integrated agriculture better prospects from an economical point of usage, as the cost of plant protection is closely connected to the quantity of the applied EPNs.

However, it is also important to note that results from laboratory tests are not always comparable to field testing (Cantelo and Nickle, 1992) as the functioning of EPNs in the open is influenced by an extensive list of factors. In one relevant study, the 100 % efficacy rate of *S. carpocapsae* in controlling Colorado potato beetle adults, pupae, and larvae in the laboratory manifested as only a 31 % reduction rate in this pest population when the test was repeated outdoors (Stewart *et al*., 1998). Some further results from studies on the activity of EPNs on other species of beetles (Toepfer *et al*., 2005; Trdan *et al*., 2006) have also shown that these agents could be an effective alternative to insecticides. Some

research has also shown that with proper application techniques and right timing as regards the insect developmental stage, we can reach almost the same results as with the use of insecticides (Schroer *et al*., 2005). Our current aim is to continue the present research under field conditions as soon as possible. Now that the use of *S. feltiae*, *S. carpocapsae*, *S. kraussei*, and *H. bacteriophora* is allowed in Slovenia – namely, due to the fact that recently all of them became an indigenous species in our country (Laznik *et al*., 2008; Laznik *et al*., 2009abc) – there are no longer any legal obstacles to carrying out field experiments with these biological control agents.

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